Molecular study for Acromegaly and the growth hormone-insulin-like growth factor-1 axis

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Abstract

The current study was conducted with the aim of studying the molecular relationship between acromegaly (local gigantism) and the human growth hormone axis - insulin-like growth factor-1, as the gene responsible for growth hormone is part of a cluster that contains a group of 4 genes that have a relationship affecting the erasure of growth hormone - Insulin-like growth factor-1. The current study indicated that there is a relationship between variations in the genetic loci rs1423321088, rs1410860750, rs5388 and the occurrence of gigantism, and that the heterozygote variant is the most prevalent. We also found that the C allele was associated with a lower risk of acromegaly compared to other alleles. The statistical analysis also showed a significant correlation between the genotype (TT vs CC vs TC vs GA) and a higher risk of acromegaly. The study also indicates that the studied genetic loci are responsible for important sites on the growth hormone protein. And the variable frequencies of the allele between different races, geographical regions and populations led to conflicting results regarding the polymorphism of genetic loci.

INTRODUCTION

Gigantism and Acromegaly

The rates at which children grow and the final height that is reached in adulthood are governed by environmental factors that interact with many genetically controlled traits, that is, height is determined by a complex interaction between genetic and environmental factors. (1,2) and pituitary gigantism is a rare but highly important subset of hypertensive patients. This disease is caused by chronic growth hormone and insulin-like growth factor-1 secretion from a pituitary adenoma from a pituitary Somatotroph. Adenoma A hyperplasia that occurs in childhood or adolescence that forms before the epiphysis closes and this hormonal secretion can lead to a severe elevation in the final adult height. Genetic causes have been identified in about 50% of cases, such as mutations in the AIP gene or duplications of chromosome Xq26.3 in hypertrophy syndrome. X-linked thyroid (3). The main difference between them is the condition of the epiphyseal growth plates at the time of growth hormone hypersecretion; Gigantism occurs during infancy when the growth plates have not yet fused, while acromegaly occurs after epiphyseal fusion (4). However, both disorders can occur in the setting of known genetic syndromes, including familial isolated pituitary adenoma and X-linked acrogiantism. Carney complex, multiple endocrine neoplasia (type 1), McCune-Albright syndrome, paraganglioma, pheochromocytoman, pituitary adenoma association, neurofibromatosis type 1 type1, which is the failure to suppress growth hormone to less than 1 μg/L after stimulation. The most common cause of both gigantism and acromegaly is a growth hormone (GH) secreting pituitary adenoma (PA), also called a pituitary neuroendocrine tumor (PA). PitNET),

which accounts for approximately 9-13% of all pituitary adenomas (PA). (5) . One of the most common diseases associated with excess growth hormone is localized gigantism, which is mostly caused by excess growth hormone (GH) and thus leads to the hallmarks of acromegaly, soft tissue hypertrophy, insulin resistance, cardiovascular and gastrointestinal diseases. Patients may exhibit clinical features, such as abnormal growth. In the hands, feet, facial features, and enlarged organs (6), females are affected slightly more (1: 1.24) than males, and the peak of diagnosis is during the fifth decade of life (22), and there are no accurate data available on the ratio Percentage of unaffected acromegaly patients who have a congenital genetic line (germline or mosaic) (7)

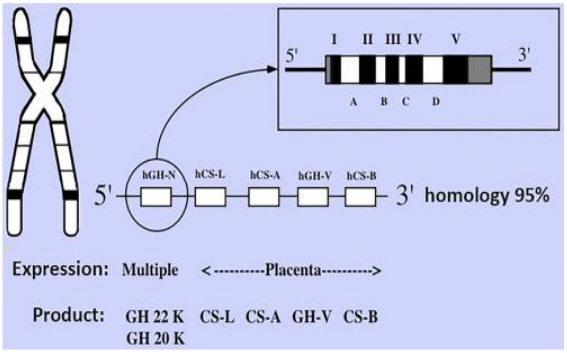
Polydactyly

Polydactyly, also known as hyperdactyly or hexadactyly, is the most common genetic abnormality of the limbs characterized by extra fingers or toes, with associated morphological phenotypes as part of a syndrome (polydactyly syndrome) or may occur as a separate event (non-syndromic polydactyly). Fingers). Broadly, non-syndromic polydactyly has been classified into three types, viz.; preaxial polydactyly (radial), central polydactyly (axial), and postaxial polydactyly (ulnar). It is often inherited as an autosomal dominant with variable penetrance and results from defects in anterior and posterior patterning for the development of limbs,To date at least 10 loci and six genes causing non-syndromic polydactyly have been identified, including ZNF141, GLI3, MIPOL1, IQCE, PITX1 and GLI1 (8).

GH Gene

The human growth hormone gene is a cluster gene. The gene is located alongside four other related genes as it intersperses in the same direction of transcription. It belongs to the GH gene cluster covering a distance of about 65 kb on the long arm of chromosome 17 (q22-24) (23) and contributes three types of proteins: pituitary GH, which consists of at least two isoforms of which one is 22 kDa and the other 20 kDa, placental GH, which also displays isoforms, and chorionic somatotropin (CSH). Figure (1)

Figure (1) Expression products of the hGH gene family share 95% similarity between them. The major GH form corresponds to a 22 kDa GH-N is expressed not only at the pituitary level but also in many different peripheral cells and tissues (9).



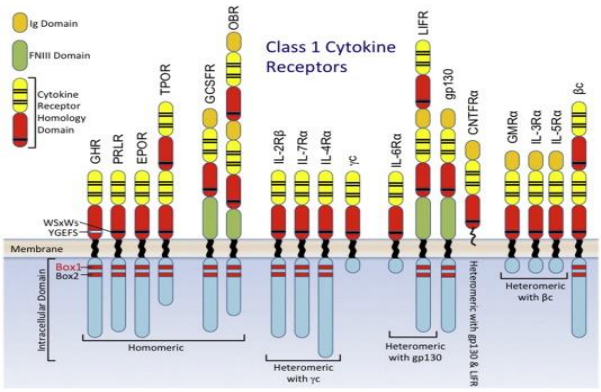
The pituitary growth hormone results from the expression of the GH-1 gene (GH-N) largely in the anterior pituitary gland and also with a minor extent in the brain, immune cells, reproductive tract (breast, ovary, testis, prostate), gastrointestinal tract and lungs (10), while placental GH is produced by expression of the GH-2 gene ((GH-V) and is contributed to CSH by expression of CSH-1 and CSH-2 genes exclusively by the placenta in females during pregnancy on the other hand, Growth hormone expression and synthesis in tissues other than the pituitary gland, including the eye, have recently been described (11) This variation in expression is mediated by elements of the locus control region (LCR) and epigenetic regulation. (12) The position of the GH-1 gene is at

17q23.3 and its length is 1,637 bp. It consists of five coding regions and four non-coding regions. In return, the GH-2 gene differs from the basic chain of the GH-1 growth hormone gene, as it contains changes in the binding sites of the second intron and contains 13 amino acids. During the second half of pregnancy it replaces pituitary GH in the maternal circulation. CSH-1, CSH-2, and GH-2 can cross the placenta and all contribute to fetal development, but only GH-1 stimulates growth later on (13) as the protein encoded by the GH-gene is 1 is a member of the somatotropin/prolactin family of hormones that plays an important role in controlling growth. Mutations or deletions in this gene lead to growth disorders such as growth hormone deficiency and short stature (14)

Growth hormone receptors

GH exerts physiological actions primarily through alterations in gene expression, which are initiated by activation of transmembrane receptors and the resulting activation of associated JAK2 (Janus kinase 2), Lyn, a member of the Src family of kinases, and the growth hormone receptor (GHR) is a member of the cytokine receptor family. From the first category, as in Figure (2), which includes more than 30 receptors, and it is well known for regulating growth and was previously thought to be present only in the liver, muscles, and adipose tissue. It is now known that growth hormone receptors are distributed everywhere, in accordance with With many multidirectional actions of growth hormone (15a). And the receptors are made of one or more pieces of proteins and include a long piece of protein consisting of 638 amino acids and are usually found on the cell wall and are a link between the outer and inner surface of the cell and have an outer end and an inner end, the N-terminal domain consists of amino acids 141-19 In the extracellular domain (ECD), the C-terminus of amino acids 146-264 in the intracellular domain (ICD) is separated by a hinge region of four amino acids (16).

Figure (2) domain structures of class I cytokine receptors (15-b)



This protein is produced by the GHR gene from the short arm of chromosome 5 (p13.1-p125). It is a gene containing 18 coding regions and is expressed in the liver, fat and other body tissues. Mutations in this gene have been associated with Laron syndrome, also known as hormone sensitivity syndrome. Developmental disorder (GHIS), a disorder characterized by short stature in humans and rabbits (NCBI). That after interacting with its membrane receptor, GH is internalized with its receptor (GHR) via the endogenous pathway and that this mechanism allows transport of both GH and GHR into the nucleus where it promotes transcription of A number of genes and on this basis, the detection of GHR in the cell nucleus indicates a previous GH-GHR interaction at the cell membrane level (17). GH signaling is mediated by JAK2 phosphorylation. Activation of the receptors involves movements initiating the hormone within the homodimer paired peptide chain of the receptor, rather than simple receptor dimers. The binding of the hormone reorganizes the orientation of the two receptors by relative rotation and by proximal position above the cell membrane. This is a

consequence of positioning. Asymmetric binding sites on the hormone and binding shifts parallel receptor transmembrane domains into a rotating cross orientation, which results in separation of the bottom of the transmembrane helices. Since JAK2 binds to the proximal Box1 element of the inner membrane, Activation of the receptor results in the separation of two associated JAK2s, and in particular the removal of the inhibitory kinase domain from the kinase domain of the other JAK2 (and vice versa). This positions the two kinase domains for transient activation and initiates tyrosine phosphorylation of the cytoplasmic receptor domain and other substrates such as STAT5, a major transcription factor that mediates most genomic actions of GH. GH signaling is mediated by JAK2-independent signals including the Src/Src pathways. ERK (S42 (15). Not only is GH signaling dependent on the amounts of GH in the circulation, but also on the levels of GHR at the cell surface and that the response (sensitivity) of cells to growth hormone is dynamically regulated reflecting the balance of endocytosis of receptors versus degradation and translocation of newly synthesized receptors. to the plasma membrane (16) The importance of the growth hormone receptor is not limited to regulating growth, but also has many other important biological functions, including regulating metabolism and controlling physiological processes related to the liver, heart, blood vessels, kidneys, digestive system, and reproductive system (18). In addition, Dysregulation of GHR signaling is associated with various diseases and chronic conditions such as acromegaly, cancer, aging, metabolic diseases, fiber, inflammation and autoimmunity making GHR an attractive therapeutic target against dwarfism (eg, isolated growth hormone deficiency, IGHD), gigantism, lipodystrophy and certain types of cancer (16).

Materials and Methods

The study samples were collected by drawing venous blood for (22) blood samples from individuals with gigantism and (5) samples from healthy people who were used as a comparison sample (control group) from the reviewers in a special laboratory for pathological analyzes - Ramadi. Doctors specializing in hormonal diseases in the Women's and Children's Hospital - Ramadi / Growth Hormone Unit and laboratory tests, as well as relying on clinical diagnosis represented by physical symptoms and family history of the disease.

study Molecular

DNA extraction method

The genomic DNA was extracted from the 22 individuals with localized gigantism and a control group according to the method attached to the diagnostic kit of the American company Geneaid, after 2 ml of venous blood was withdrawn by means of a sterile medical syringe, and then the blood was transferred into tubes containing an anticoagulant. The EDTA tube was gently inverted several times, then kept in ultra-freezing (-20 C) until the extraction process.

Agarose gel electrophoresis of DNA

The solutions and buffers used for electrophoresis were prepared according to (19,24) .

Design Of Primers

Three pairs of specialized primers were designed for each SNP designed by the researcher by extracting the GH1 gene sequence from the NCBI website

www.ncbi.nlm.nih.gov, and the Primer 3 plus program http://www.bioinformatics.nl/cgi was used. -bin, to design 3 primers according to the T-ARMS–PCR system. The primers are in a lyophilized form from the American company Pioneer, and then they were dissolved using distilled water Molecular grade water to prepare a stock solution with a concentration of pmoles / ul100, from which a dilution of pmoles 10 was used to conduct the polymerase chain reaction (PCR) experiments

Table (1) the specialized prefixes used in this study with their sequences and their expected size

	Primer		Sequence	Tm (°C)	Product size
1	***KS – P1 rs5388***	**FO-O**	5-CCCCCATCAGCGTTTGGATG-3	61.39	104bp
		RE-O	5-CCTCAGGAGTGTCTTCGCCA-3		
		FO-I	5-TCCTTTAGGAGGTCATAGAC-3	61.54	
		RE-I	5-CGGCGCCTCTGACAGCAACT-3		
2	***KS – P2 rs1423321088***	**FO-O**	5-TTCCTGAAGCAGTAGAGCAG-3	56.95	95bp
		RE-O	5-CAGATCTTCAAGCAGACCTA-3		
		FO-I	5-GTTCTTGAGTAGTGCGTCAT-3	54.19	
		RE-I	5-GACACAAACTCACACAACGC-3		
3	***KS – P3 rs1410860750***	**FO-O**	5-CAGGGCCAGGAGAGGCACTG-3	64.69	141bp
		RE-O	5-CTCTACTGCTTCAGGAAGGA-3		
		FO-I	5-GATGCCACCCGGGCAGCTAG-3	55.98	
		RE-I	5-TGGAGGGCAGCTGTGGCTTT-3		

Results

Estimation of hGH concentration in serum

The hormone was estimated in the blood serum using the ELISA technique, according to the method attached to the measurement kit prepared by Monobind Inc.

Estimation of insulin-like growth factor (IGF-1) concentration in blood serum

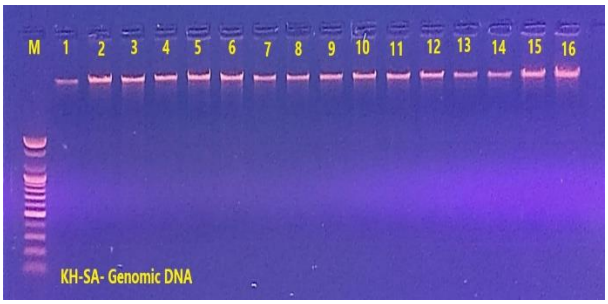
The hormone was estimated in the blood serum using the ELISA technique, according to the method attached with the measurement kit provided by INVITROGEN.

Molecular study

DNA extraction

The current study included the extraction of DNA from blood samples of (22) study individuals, as well as from (5) control samples, depending on the method of extracting DNA from total blood, which was described in the chapter on materials and methods of work, and purity was measured. And the concentration of the samples was mediated by a (Nano Drop) device, as the purity and concentration were within the ideal limits and suitable for the (tetra-primer ARMS-PCR) reaction. Agarose with a concentration of (1%).

Fig. (3) DNA extracted and carried over on an agarose gel with a concentration of 1% 5 vol. /cm for 1:15 hour.



Electrophoresis results for PCR products with a single allelic polymorphism of rs5388

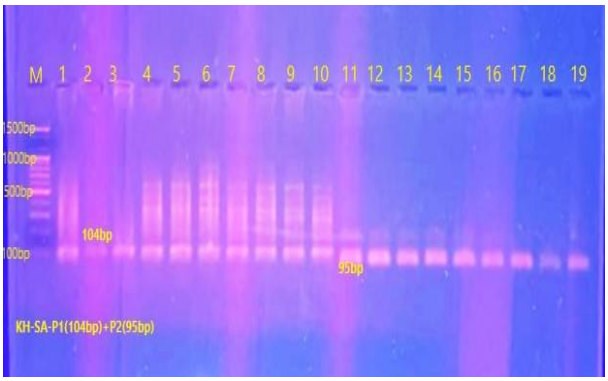
The results of the current study show, Table (2) Figure (4), the occurrence of polymorphism in the forms or sites of the nitrogenous bases (SNPs), as the site rs5388 showed the occurrence of polymorphism in the base site (C (104bp)), in which the number of appearance of the bands reached 20 (91%) in the study sample, compared to 5 (100%) in the control sample, while the study showed the occurrence of polymorphism in the above nucleotide form, as it showed two additional forms, TT (80) and CT (80 + 104), with the number of occurrences and frequency It reached 1 (4.5%) and 1 (4.5%), respectively, compared to the number of times it appeared in the control sample, which amounted to 0 (0%).

Table (2) Number of registered genotypes according to the location of the nitrogenous bases

P1	Ge.(104)	Ge.(80+104)	Ge.(80)	SNP	ت
Patients	CC=20	CT=01	TT=01	(*rs5388*)	1
Control	CC=5	CT=00	TT=00		
P2	Ge.(95)	Ge.(95+80)	Ge.(80)		
Patients	TT=11	TC=10	CC=01	(*rs1423321088*)	2
Control	TT=01	TC=00	CC=04		

P3	Ge.(141)	Ge.(141+100)	Ge.(100)		
Patients	AA=03	AG=15	GG=04	(*rs1410860750*)	3
Control	AA=03	AG=01	GG=01		

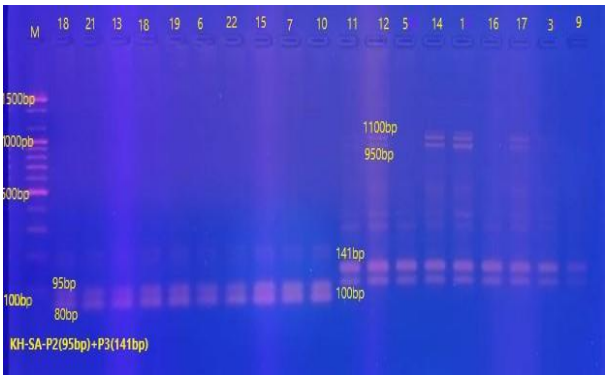
Figure (4) the product of electrophoresis of single nucleotides at position rs5388 and rs1423321088 on agarose gel for some study samples with a concentration of 1.5% 5 vol. /cm for 1:15 hour.



Electrophoresis results for PCR products single-allelic polymorphisms rs1423321088

The results of the current study also showed tables (2) Figure (5) the occurrence of multiple forms or sites of nitrogenous bases (SNPs), as the site rs1423321088 showed the occurrence of multiplex in the base site (T (95bp)), in which the number of bands appeared 11 (50%) in the study sample, compared to 1 (20%) in the control sample, while the study showed the occurrence of polymorphism in the above nucleotide form, as it showed two additional forms, C (80bp) and TC (95 + 80bp), with the number of occurrences Its frequency was 10 (45.5%) and 1 (4.5%), respectively, compared to the number of times it appeared in the control sample.

Figure (5) Electrophoresis product of single nucleotide at position rs1423321088 and rs1410860750 on agarose gel for some study samples with a concentration of 1.5% 5 vol. /cm for 1:15 hour.



Electrophoresis results for polymerase chain reaction products single-allelic polymorphisms rs1410860750

The results of the current study, Table (2), Figures (4,5), indicated the occurrence of polymorphism in the forms or sites of the nitrogenous bases (SNPs), as the site rs1410860750 showed the occurrence of polymorphism in the base site (A (141bp)), which reached the number of appearance of bundles There are 3 (13.6%) in the study sample, compared to 3 (60%) in the control sample, while the study showed the occurrence of polymorphism in the above nucleotide form, as it showed two additional forms, G (100bp) and GA (141 + 100bp), and a number of times Their occurrence and frequency amounted to 4 (18.2%) and 15 (68.2%), respectively, compared to the number of times they appeared in the control sample, which amounted to 1 (20%) and 1 (20%). The genetic variations of

the human GH1 gene are one of the important issues that many researchers have addressed (Giordano, M.et al, 1997; Horan, M.et al, 2003; Adkins, R, et al, 2005), as they indicated that more than 50% Among the variations occurred within the initiator region and the 5′ UTR region, and in a study conducted in Egypt on people suffering from terminal gigantism, it was found that the genotypes of the "A" allele were significantly high and the relationship was statistically significant between the "A" allele and low levels of GH in the group The study showed that the use of SNP as a biomarker of status has an important role (20). While a study conducted on the Turkish population indicated that the frequency of the "T" allele was 83%, while the "A" genotype was not found. This variable relationship between the SNP with the variance in GH concentration may be due to several reasons, the most important of which is that the SNP alone may not have an effect on GH levels but may be associated with disequilibrium with other effective genetic polymorphisms (21). In our current study, the distribution of the genotype of the studied genetic sites between the study and control groups shows that the heterozygote variant is the most prevalent. We also found that the C allele was associated with a lower risk of acromegaly compared to other alleles, although the statistical significance was limited because there are a limited number of samples, the statistical analysis showed a significant association between the genotype (TT vs CC vs TC vs GA) and the high risk of acromegaly. The study also indicates that the studied genetic loci are responsible for important sites on the growth hormone protein. Therefore, we tried to determine whether the variation between polymorphisms at loci constitutes a risk factor for acromegaly. In addition, the variable frequencies of the allele between different races, geographic regions and populations may lead to results Conflicting regarding the polymorphism of genetic loci, although our study has some advantages. Being one of the first studies to investigate cases of acromegaly that studies the relationship between polymorphisms of genetic loci and the risk of acromegaly, this study faced many obstacles and limitations. The samples were chosen far from randomness, which may lead to selection bias. On the other hand, the low number of participants in the study added new difficulties to the role of comparisons between alleles, which caused statistical limitations.

Reference

1-Jelenkovic, A., Sund, R., Hur, Y. M., Yokoyama, Y., Hjelmborg, J. V. B., Möller, S., ... & Silventoinen, K. (2016). Genetic and environmental influences on height from infancy to early adulthood: An individual-based pooled analysis of 45 twin cohorts. Scientific reports, 6(1), 1-13.

2-Shrine, N., Tobin, M. D., & Wain, L. V. (2017). CNV-association meta-analysis in 191,161 European adults reveals new loci associated with anthropometric traits.

3-Beckers, A., Petrossians, P., Hanson, J., & Daly, A. F. (2018). The causes and consequences of pituitary gigantism. Nature Reviews Endocrinology, 14(12), 705-720.

4-Makri, A., and Lodish, M. (2019). Gigantism and Acromegaly. In Pituitary Disorders of Childhood (pp. 121-139). Humana Press, Cham.

5-Hannah-Shmouni, F., Trivellin, G., and Stratakis, C. A. (2016). Genetics of gigantism and acromegaly. Growth hormone & IGF research, 30, 37-41.

6-Lu, M., Flanagan, J. U., Langley, R. J., Hay, M. P. and Perry, J. K. (2019). Targeting growth hormone function: strategies and

therapeutic applications. Signal transduction and targeted therapy, 4(1), 1-11.

7-Gadelha, M. R., Kasuki, L.,and Korbonits, M. (2017). The genetic background of acromegaly. Pituitary, 20(1), 10-21.

8-Umair, M., Ahmad, F., Bilal, M., Ahmad, W., & Alfadhel, M. (2018). Clinical genetics of polydactyly: an updated review. Frontiers in genetics, 9, 447.

9- Devesa, J., Almengló, C., & Devesa, P. (2016). Multiple effects of growth hormone in the body: is it really the hormone for growth?. Clinical Medicine Insights: Endocrinology and Diabetes, 9, CMED-S38201.

10- Melmed, S., Polonsky, K. S., Larsen, P. R., & Kronenberg, H. M. (2015). Williams textbook of endocrinology E-Book. Elsevier Health Sciences.

11 -Pérez-Ibave, D. C., Garza-Rodríguez, M. L., Pérez-Maya, A. A., Rodríguez-Sánchez, I. P., Luna-Muñoz, M., Martínez-Moreno, C. G., ... & Barrera-Saldaña, H. A. (2019). Expression of growth hormone and growth hormone receptor genes in human eye tissues. Experimental Eye Research, 181, 61-71.

12-Murray, P. G., Higham, C. E., & Clayton, P. E. (2015). 60 years of neuroendocrinology: the hypothalamo-GH axis: the past 60 years. Journal of Endocrinology, 226(2), T123-T140.

13-Insensitivity, G. H., & Variant, N. (2018). Growth Disorders. Pediatric Endocrinology: A Practical Clinical Guide, 2.

14-National Center for Biotechnology Information.

15-a-Waters, M. J. (2016). The growth hormone receptor. Growth Hormone & IGF Research, 28, 6-10.

16-Strous, G. J., Almeida, A. D. S., Putters, J., Schantl, J., Sedek, M., Slotman, J. A., ... & Mol, J. A. (2020). Growth hormone receptor regulation in cancer and chronic diseases. Frontiers in endocrinology, 11, 597573.

15-b-Waters, M. J., Shang, C. A., Behncken, S. N., Tam, S. P., Li, H., Shen, B., & Lobie, P. E. (1999). Growth hormone as a cytokine. Clinical and Experimental Pharmacology and Physiology, 26(10), 760-764.

17-Garcia-Barros, M., Devesa, J., & Arce, V. M. (2000). Proteolytic processing of human growth hormone (GH) by rat tissues in vitro: influence of sex and age. Journal of endocrinological investigation, 23(11), 748-754.

18-Dehkhoda, F., Lee, C. M., Medina, J.,and Brooks, A. J. (2018). The growth hormone receptor: mechanism of receptor activation, cell signaling, and physiological aspects. Frontiers in endocrinology, 9, 35.

- 19-Dillon, J.R. Nasim, A. and Nestmann, E.R. (1985). Recombinant DNA Methodology . John Wiley and Sons , USA.

20-Esmaiel NN, Fayez AG, Thomas MM, et al. The association of +1150A polymorphism with low GH level in isolated growth hormone deficiency (IGHD) patients. Gene Rep. 2019;14(December):118-123

21-Elif Y, Oya E. , Servet E. ,Adal A. Nur B., Metin Y., Ayhan D.(2021) Common Polymorphisms of Growth Hormone: Growth Hormone Receptor Axis in Turkish Children with Short Stature, Turk Arch Pediatr 2022; 57(2): 160-167

22-Lavrentaki, A., Paluzzi, A., Wass, J. A., & Karavitaki, N. (2017). Epidemiology of acromegaly: review of population studies. Pituitary, 20(1), 4-9.

23-Jin, Y., Vakili, H., Liu, S. Y., Menticoglou, S., Bock, M. E., & Cattini, P. A. (2018). Chromosomal architecture and placental expression of the human growth hormone gene family are targeted by pre-pregnancy maternal obesity. American Journal of Physiology-Endocrinology and Metabolism, 315(4), E435-E445.

24-Ausuble, F., Brent, R., Kingston, D., Moore, J., Smith, J. Seidman and Struk, K. (1987).Current protocols in Molecular Biology.John Wiley and Sons , inc., New York